Topical Review

Physiological roles of ATP-sensitive K^+ channels in smooth muscle

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Potassium channels that are inhibited by intracellular ATP (ATP_i) were first identified in ventricular myocytes, and are referred to as ATP-sensitive K^+ channels (i.e. K_{ATP} channels). Subsequently, K^+ channels with similar characteristics have been demonstrated in many other tissues (pancreatic β-cells, skeletal muscle, central neurones, smooth muscle). Approximately one decade ago, K_{ATP} channels were cloned and were found to be composed of at least two subunits: an inwardly rectifying K^+ channel six family (Kir6.x) that forms the ion conducting pore and a modulatory sulphonylurea receptor (SUR) that accounts for several pharmacological properties. Various types of native K_{ATP} channels have been identified in a number of visceral and vascular smooth muscles in single-channel recordings. However, little attention has been paid to the molecular properties of the subunits in K_{ATP} channels and it is important to determine the relative expression of K_{ATP} channel components which give rise to native K_{ATP} channels in smooth muscle. The aim of this review is to briefly discuss the current knowledge available for K_{ATP} channels with the main interest in the molecular basis of native K_{ATP} channels, and to discuss their possible linkage with physiological functions in smooth muscle.

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It is generally accepted that K^+ channel activation prevents the development of action potentials in smooth muscles, so allowing graded changes in membrane potential to regulate their tone. Several K^+ channels, with different molecular bases, contribute to the regulatory basal K^+ conductance in smooth muscle cells: (i) voltage-gated K^+ channels (Kv); (ii) large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}); (iii) small conductance Ca^{2+}-activated K^+ channels (SK_{Ca}); (iv) inward rectifier K^+ channels (Kir); (v) two-pore domain K^+ channels (TASK); (vi) stretch-dependent K^+ channels and (vii) K_{ATP} channels (Thorneloe & Nelson, 2005).

K_{ATP} channels were first identified in cardiac myocytes (Noma, 1983). Being selective for K^+ and activated by a fall in the internal concentration of ATP, K_{ATP} channel activity may confer a voltage-independent 'brake' which limits myogenic depolarization and controls myogenic reactivity. Several endogenous agonists (such as calcitonin gene-related peptide (CGRP), adenosine, etc.) activate K_{ATP} channels leading to hyperpolarization and relaxation, a response that is mimicked by treatment with K_{ATP} channel openers. In contrast, various neurotransmitters (noradrenaline (norepinephrin), 5-hydroxytryptamine (5-HT), neuropeptide Y, etc.) and vasoconstrictors (angiotensin II, endothelin-1, etc.) inhibit K_{ATP} channels leading to depolarization and contraction (Quayle *et al.* 1997). Thus, modulation of K_{ATP} channels allows the contribution of native K_{ATP} channels to be finely tuned, so regulating the contractility of smooth muscle.

K_{ATP} channels are octameric complexes of pore-forming and modulatory subunits

Recent progress in defining the molecular basis of K_{ATP} channels in different tissues indicates that there is functional diversity which results from cell-specific expression of different subunit proteins (Aguilar-Bryan & Bryan, 1999). When K_{ATP} channels were cloned (Inagaki *et al.* 1995), they were found to contain four pore-forming, inwardly rectifying channel subunits (Kir6.x) and four modulatory sulphonylurea receptor subunits (SUR.x) that are members of the ATP-binding cassette (ABC) super-family of proteins (Fig. 1). To date, two Kir6 isoforms, Kir6.1 and Kir6.2, and two SUR isoforms, SUR1 and SUR2, have been identified (Aguilar-Bryan & Bryan, 1999). Kir6.x subunits have two transmembrane domains, M1 and M2, cytoplasmic N- and C-termini and

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Table 1. Smooth muscle-type recombinant K_{ATP} channels

Subunit composition	Conductance (140 mm K^+)	Native channel	Reference
Kir6.2–SUR2B	80 pS	K_{ATP} channel	Isomoto *et al*. 1996
Kir6.1–SUR2B	33 pS	K_{NDP} channel	Yamada *et al*. 1997

a pore-forming loop typical of inward rectifier K^+ channels (Aschroft & Gribble, 1998; Aguilar-Bryan & Bryan, 1999; Seino, 1999). Channels containing Kir6.1 have a unitary conductance of ∼35 pS, whereas for Kir6.2 channels this is ∼70 pS in symmetrical 140 mM K^+ conditions. Alternative splicing of exon 38 results in two species of SUR2, i.e. SUR2A (Inagaki *et al.* 1996) and SUR2B (Isomoto *et al.* 1996). The 42 amino acid residues located in the carboxyl-terminal end of SUR2B is divergent from that of SUR2A but highly homologous to that of SUR1 (Isomoto *et al.* 1996). SURs possess large cytoplasmic domains containing two conserved nucleotide binding folds (NBFs), NBF1 and NBF2, with Walker A and B motifs (Fig. 1). The endoplasmic reticulum (ER) retention motifs are present in the cytoplasmic domains of the Kir6 and SURs; they preclude cell surface expression unless both subunits are present (Zerangue *et al.* 1999).

Kir6.x–SUR.x combination

Different combinations of Kir6.x and SUR.x isoforms/variants yield tissue-specific K_{ATP} channel subtypes with different features and distinct functional properties. In functional expression studies, it is accepted that SUR1–Kir6.2 forms the pancreatic β-cell K_{ATP} channel and that SUR2A–Kir6.2 forms the cardiac K_{ATP} channel (Aschroft & Gribble, 1998; Aguilar-Bryan & Bryan, 1999). However, the molecular identity of smooth muscle-type K_{ATP} channels has not been established with the same certainty. Two types of smooth muscle-type K_{ATP} channels have been cloned and identified (Table 1), namely Kir6.2–SUR2B channels (Isomoto *et al.* 1996) and Kir6.1–SUR2B channels (Yamada *et al.* 1997).

Isomoto *et al.* (1996) have succeeded in isolating a cDNA that encodes a novel variant of SUR, termed SUR2B. Coexpression of SUR2B with Kir6.2 produces activity

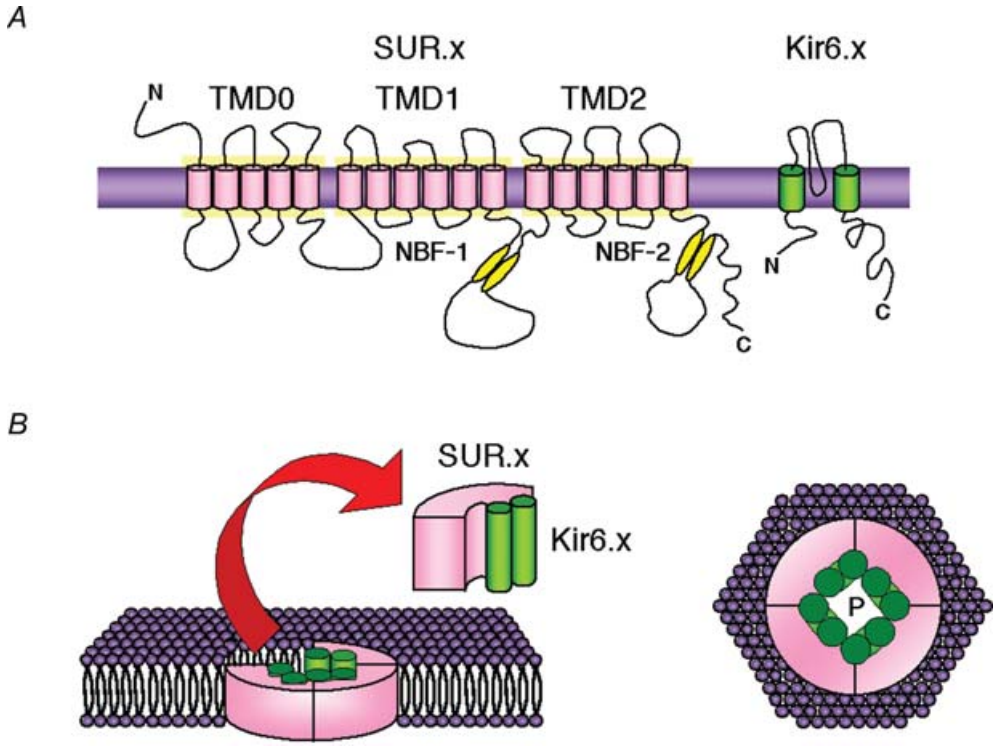

Figure 1. Schematic illustration of the predicted topologies of Kir6.x and SUR.x subunits and the assembly of these subunits into a K_{ATP} channel
The transmembrane domain model proposed by Tusnady *et al.* (1997) is given. NBF-1 and -2 represent the two nucleotide-binding folds with Walker A and B consensus motifs. N and C indicate the N and C termini of the proteins (adapted from Cole & Clément-Chomienne, 2003). P, channel pore; Kir6.x, inwardly rectifying K^+ channels; SUR.x, sulphonylurea receptors; TMD, transmembrane domain.

Table 2. Native K_{ATP} channels in visceral and vascular smooth muscles (RT-PCR analysis)

Smooth muscle	Transcript				Subunit composition	Conductance (140 mM K^+)	Reference
	Kir6.1	Kir6.2	SUR1	SUR2B			
Murine colon	–	+	–	+	Kir6.2–SUR2B	27 pS	Koh *et al*. 1998
Guinea-pig stomach	+	+	–	+	Kir6.1–SUR2B	37 pS	Sim *et al*. 2002
Pig urethra	+	+	+	+	Kir6.1–6.2–SUR1 Kir6.1–6.2–SUR2B	43 pS	Teramoto *et al*. 1997*b* Teramoto *et al*. 2000 Yunoki *et al*. 2002
Cultured human	+	–	–	+	Kir6.1–SUR2B	28 pS	Cui *et al*. 2002

which is enhanced by K_{ATP} channel openers. Moreover, RT-PCR analysis indicates that transcripts for SUR2B are widely distributed in various smooth muscles (Isomoto *et al.* 1996). These results suggest that Kir6.1–SUR2B is a native smooth muscle-type K_{ATP} channel. However, the electrophysiological properties of Kir6.1–SUR2B channels are not consistent with those of all K_{ATP} channels detected in several smooth muscles (Quayle *et al.* 1997).

Yamada *et al.* (1997) reported that reconstituted Kir6.1–SUR2B channels resemble nucleotide diphosphate (NDP)-sensitive K_{NDP} channels in some vascular smooth muscle (Beech *et al.* 1993; Zhang & Bolton, 1996; Cole *et al.* 2000). Characteristically they demonstrate: (1) a unitary conductance of 33 pS with no voltage dependency; (2) channel activity is enhanced by K_{ATP} channel openers with burst-like openings, which are inhibited by glibenclamide; (3) no channel opening is observed in the absence of K_{ATP} channel openers; (4) internal application of NDPs can restore activity even after run-down is complete; (5) ATP_i appears to be required to maintain activity in the presence of Mg^{2+}, even though channel current–voltage relationships are linear and show no inward rectification in the presence of Mg^{2+}. Furthermore, Kir6.1–SUR2B channels are not suppressed by physiological concentrations of ATP_i. Thus, it is somewhat uncertain whether or not Kir6.1–SUR2B channels may be classified into a category of 'K_{ATP} channels', although they do resemble K_{NDP} channels.

Molecular basis of native K_{ATP} channels in smooth muscle

A point-for-point quantitative comparison between native and recombinant K_{ATP} channels is required to determine the pattern of K_{ATP} channel subunit expression in smooth muscle. In visceral and vascular smooth muscle, the molecular properties of native K_{ATP} channels have been investigated in RT-PCR analysis in addition to electrophysiological observations. Table 2 summarizes the published molecular and electrical properties of native K_{ATP} channels. Various combinations of Kir6.x and SUR.x convey the heterogeneity.

In murine colon, mRNAs of Kir6.2 and SUR2B are detected and the unitary channel conductance is 27 pS, suggesting that Kir6.1–SUR2B forms the K_{ATP} channels in this tissue (Koh *et al.* 1998). In guinea-pig stomach, the transcripts of Kir6.1, Kir6.2 and SUR2B but not SUR1 are present and channel conductance is 37 pS, suggesting that K_{ATP} channels are composed of Kir6.1 and SUR2B (Sim *et al.* 2002). In pig urethra, RT-PCR analysis demonstrates the presence of SUR1 and SUR2B transcripts and both SUR1 and SUR2B coexist as functional SUR subunits (Yunoki *et al.* 2002, 2003). The transcripts of Kir6.1 and Kir6.2 have been detected in pig urethra (Teramoto *et al.* 2000, 2003) and the conductance of urethral K_{ATP} channels is 43 pS (an intermediate conductance between that of Kir6.1 and Kir6.2; Teramoto *et al.* 1997*b*), suggesting that the nature of the pore region of urethral K_{ATP} channels differs from that of vascular K_{ATP} channels (Tomoda *et al.* 2005) and that urethral K_{ATP} channels may possess a heteromeric channel structure with the channel pore composed of Kir6.1 and Kir6.2 subunits (Teramoto *et al.* 2003). In smooth muscle-type K_{ATP} channels, it still remains elusive as to which molecular factor(s) may modify the expression of K_{ATP} channel components (such as Kir6.x and SUR.x) in cell plasma membrane. On the other hand, in Kir6.2–SUR1 (i.e. pancreatic β cell-type K_{ATP} channels), trafficking of K_{ATP} channel complexes out of the ER is controlled by a tri-peptide Arg-Lys-Arg (RKR) retention/retrieval signal present in each of the Kir6.2 and SUR1 subunits (Zerangue *et al.* 1999). Upon successful assembly of SUR and Kir into an octameric complex, the -RKR- motifs are concealed to allow the channel to translocate from the ER to the Golgi, where the sugar moiety on SUR1 is modified before further translocation to the plasma membrane (Raab-Graham *et al.* 1999; Zerangue *et al.* 1999; Conti *et al.* 2002). Thus, in Kir6.2–SUR1, the -RKR- trafficking signal provides a quality control mechanism to prevent individual subunits as well as incompletely assembled channel complexes from trafficking to the cell surface.

In cultured human pulmonary arterial smooth muscle cells, the expression of Kir6.1 and SUR2B mRNAs has been reported (Cui *et al.* 2002). Since the unitary conductance is 28 pS, Kir6.1–SUR2B is likely to be the predominant

isoform of the native K_{ATP} channel, possessing ATP_i sensitivity (Cui *et al.* 2002). In contrast, Kir6.1–SUR2B channels expressed in HEK-293 cells are entirely insensitive to ATP_i (≤ 5 mM). It is not certain whether or not this discrepancy may be due to cultured smooth muscle cells or different recording conditions. Cui *et al.* (2002) suggest that the expression of the recombinant K_{ATP} channels in HEK-293 cells may alter ATP_i sensitivity, changing basal phosphorylation states since the activity of native K_{ATP} channels in smooth muscle cells is readily modulated by several kinases (PKA, Wellman *et al.* 1998; PKC, Bonev & Nelson, 1993; and tyrosine kinase, Hatakeyama *et al.* 1995). Alternatively, this discrepancy is related to other factors governing the regulation of K_{ATP} channels in the native environment.

Recently, a gene-targeting strategy to generate mice with disrupted muscle-specific K_{ATP} channel regulatory subunits has been carried out to improve the understanding of the role of K_{ATP} channels (reviewed by Seino & Miki, 2004). Since Kir6.1-containing K_{ATP} channels are involved in regulation of vascular tonus (Li *et al.* 2003), it would be of interest to investigate the functional properties of vascular smooth muscle in the Kir6.1 null mouse. Furthermore, it has been also reported that the Kir6.1 null mouse is a model of variant angina pectoris (i.e. Prinzmetal's angina or spontaneous angina pectoris) in human by disruption of the gene encoding Kir6.1 (Miki *et al.* 2002). Miki *et al.* (2002) suggest that smooth muscle-type K_{ATP} channels are likely to be defective in Kir6.1 null mice, concluding that Kir6.1 is a constituent of smooth muscle-type K_{ATP} channels on the plasma membrane of vascular smooth muscle.

In summary, native K_{ATP} channels in smooth muscle show considerable heterogeneity in several notable respects. Significantly, the functional expression studies also show that heteromultimerization readily occurs between Kir6.1 and Kir6.2, producing functional recombinant K_{ATP} channels that possess distinct unitary conductance values which are intermediate between the levels observed for the homomeric channels (Cui *et al.* 2001). These results suggest that multiple types of native K_{ATP} channels exist in different species and types of smooth

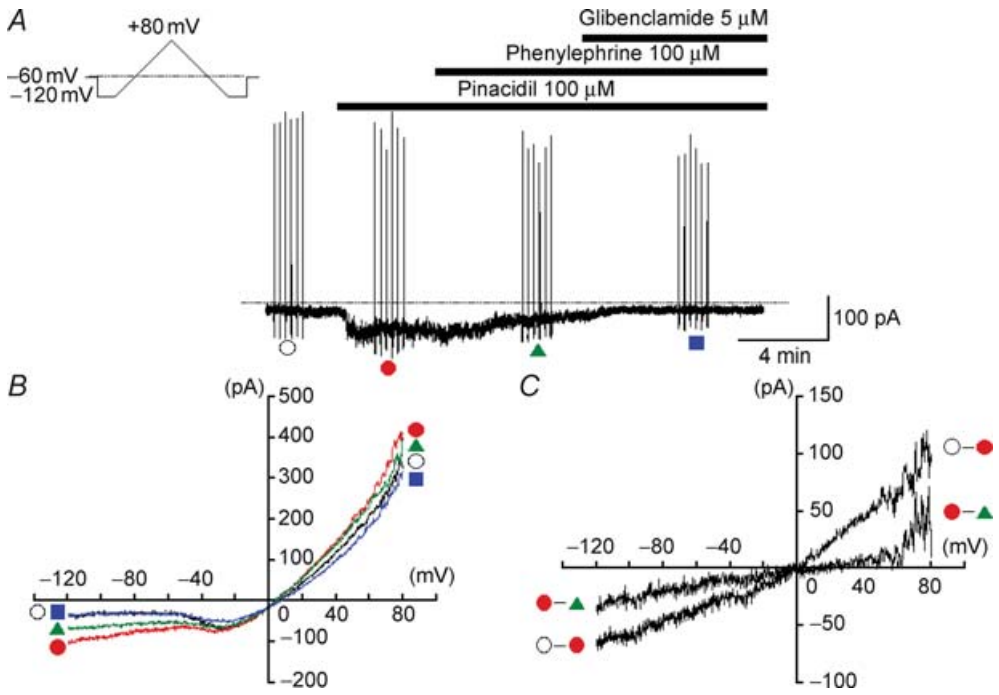

Figure 2. Effects of phenylephrine on the pinacidil-induced K_{ATP} current in smooth muscle cells dispersed from rat tail artery

Whole-cell recording, bath solution 140 mM K^+ solution, pipette solution 140 mM K^+ containing 5 mM EGTA. *A*, current trace. The vertical lines are responses to triangular ramp potential pulses of 200 mV s^{-1} from –120 mV to +80 mV, applied after an initial 300 ms conditioning pulse to –120 mV (see inset). Pinacidil (100 μM) caused an inward membrane current which was sustained. The current was inhibited by application of 100 μM phenylephrine. Additional application of glibenclamide suppressed the pinacidil-induced K_{ATP} current in rat tail artery. The dashed line indicates zero current. *B*, current–voltage relationships measured from the negative-going limb (the falling phase) of the ramp pulse. Each symbol is the same as in the current trace (*A*). The lines are mean membrane currents from the six ramps in each condition. *C*, net membrane currents. The phenylephrine-sensitive membrane current was obtained by subtraction of the membrane currents in the absence and presence of 100 μM phenylephrine when 100 μM pinacidil was present in the bath solution.

muscle and that mixed populations of Kir6.x and SURs subunits form hybrid K_{ATP} channels.

Physiological roles of the native K_{ATP} channels

K_{ATP} channels are characteristically activated by declining concentrations of ATP_i or elevated concentrations of NDPs, followed by changing the ratio of ADP/ATP. Thus, it is thought that K_{ATP} channels provide a link between cell metabolism and membrane excitability. Furthermore, K_{ATP} channels appear to be the target of a number of neuropeptides and neurotransmitters. In *in vivo* experiments, the existence of active native K_{ATP} channels in smooth muscle has been inferred through the ability of glibenclamide to produce excitation (reviewed by Quayle *et al.* 1997).

Resting membrane potential and basal tone

A number of *in vitro* studies have reported that glibenclamide ($\leq 1\ \mu$M), which blocks K_{ATP} channels, increases muscle tone and causes depolarization in vascular smooth muscle (rabbit mesenteric artery, Nelson *et al.* 1990; canine saphenous vein, Nakashima & Vanhoutte, 1995) and non-vascular smooth muscle (guinea-pig trachea, Murray *et al.* 1989; dog bronchial smooth muscle, Kamei *et al.* 1994; pig urethra, Teramoto *et al.* 1997*a*). Furthermore, *in vivo* studies also show that glibenclamide significantly increases vascular resistance and decreases arterial diameter (Quayle *et al.* 1997). Although the interpretation of theses studies solely depends on the sensitivity of glibenclamide, direct measurements of channel activity show that brief openings of native K_{ATP} channels occasionally occurred in the

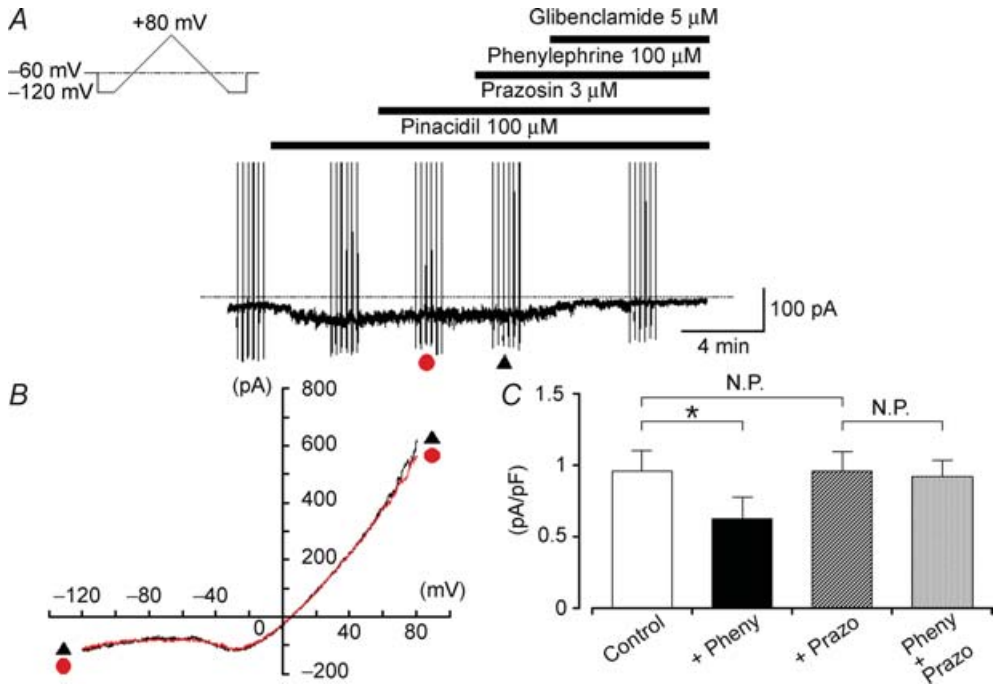

Figure 3. The phenylephrine-induced inhibition of K_{ATP} current was blocked by prazosin in rat tail artery
Whole-cell recording, bath solution 140 mM K^+ solution, pipette solution 140 mM K^+ containing 5 mM EGTA. *A*, current trace. The vertical lines are responses to triangular ramp potential pulses of 200 mV s^{-1} from –120 mV to +80 mV, applied after an initial 300 ms conditioning pulse to –120 mV (see inset). Pinacidil caused an inward membrane current which was sustained. Prazosin caused no effect on the pinacidil-induced K_{ATP} current. Similarly, additional application of phenylephrine had no effect on the pinacidil-induced K_{ATP} current in the presence of prazosin. Subsequently, application of glibenclamide suppressed the pinacidil-induced K_{ATP} current. The dashed line indicates zero current. *B*, current–voltage relationships measured from the negative-going limb (the falling phase) of the ramp pulse. Each symbol is the same as in the current trace (*A*). The lines are mean membrane currents from the six ramps in each condition. *C*, summary of the data. The open column indicates the current density (pA pF^{-1}) of the pinacidil-induced K_{ATP} currents (i.e. Control). The black column shows the current density of the pinacidil-induced K_{ATP} currents in the presence of 100 μM phenylephrine (+ Pheny). The hatched column represents the current density of the pinacidil-induced K_{ATP} currents in the presence of 3 μM prazosin (+ Prazo). The grey column indicates the current density of the pinacidil-induced K_{ATP} currents in the presence of both prazosin and phenylephrine (Pheny + Prazo). *Statistically significant difference, demonstrated using a paired *t* test ($P < 0.01$). Each column represents the relative mean value with S.D. ($n = 5$).

absence of K_{ATP} channel openers (Teramoto *et al.* 1997*a*). Presumably this is related to a low density or a low open probability of native K_{ATP} channels. These results suggest that K_{ATP} channels play important roles in regulating the resting membrane potential of several smooth muscles with a small amount of channel activity.

Interaction between cytoskeletal networks and the regulating mechanisms of K_{ATP} channels

The integrity of the microenviroment, in particular the actin filament network, surrounding K_{ATP} channel proteins may play an important role in modulating the channel activity of K_{ATP} channels (Van Wagoner & Lamorgese, 1994). DNase I, one of the actin microfilament disrupters, has been shown to stimulate the activity of K_{ATP} channels in cardiomyocytes (Terzic & Kurachi, 1996). Similarly, cytochalasin B enhanced the activity of K_{ATP} channels in native K_{ATP} channels of smooth muscle (Teramoto *et al.* 2002). The actin filament network and its related proteins might be involved in signal transaction between the inhibitory regulatory proteins and K_{ATP} channels.

Cellular pathways for K_{ATP} channel modulation

The activity of native K_{ATP} channels is increased by several vasodilators (e.g. adenosine, CGRP, prostacyclin, β agonists) which activate PKA through the formation of cyclic AMP, whereas contractile agonists (e.g. angiotensin II, endothelin-1, serotonin, noradrenaline) or α agonists (Figs 2 and 3; author's unpublished data), vasopressin, neuropeptide (Y), which activate PKC pathways, decrease the activity of native K_{ATP} channels, causing depolarization and contraction (Quayle *et al.* 1997; Cole *et al.* 2000). Although channel phosphorylation is essential for the regulation of native K_{ATP} channels in smooth muscle, the molecular basis and mechanisms by which native K_{ATP} channels are affected by PKC- and PKA-mediated phosphorylation remains unknown.

In recombinant K_{ATP} channel studies, the activation of Kir6.2–SUR1 and/or Kir6.2–SUR2A channels by PKC and PKA has been studied and potential phosphorylation sites have been identified by mutational analysis (Beguin *et al.* 1999; Lin *et al.* 2000; Manning Fox *et al.* 2004), but such studies have not been carried out on SUR2B-containing recombinant channels. PKA-mediated phosphorylation of Kir6.2–SUR1 channels was initially studied *in vitro*, and residues (i.e. Kir6.2-S372 and SUR1-S1571) were identified as potential phosphorylation sites (Beguin *et al.* 1999). Subsequent analysis suggested that these sites may not be relevant for control of gating as the SUR site is not conserved in other species, and substitution of Kir6.2-T224, but not S372, prevented PKA-mediated stimulation of gating (Lin *et al.* 2000). However, in both studies, the effects of PKA were studied in non-physiological conditions: the effect of PKA on the inhibition by ATP was assessed, but no attempt was made to evaluate the actions of PKA in the presence of MgADP. Consideration of the effects of kinase-mediated regulation of K_{ATP} channels in the presence of MgADP is essential because the relative changes in ATP sensitivity identified in these studies would be irrelevant within the normal physiological range of ATP_i of 1–5 mM. The importance of this point was illustrated in a recent study by Manning Fox *et al.* (2004) which reveals a more complex modulation involving additional sites in the presence of MgADP.

Conclusions

Several observations suggest that the Kir6.1–SUR2B channel is likely to be the predominant isoform of native K_{ATP} channel in some vascular smooth muscles. Conversely, many studies have identified the molecular properties of native K_{ATP} channels and have suggested that more than one type of K_{ATP} channel is expressed in smooth muscle. Clearly many queries remain about the nature of smooth muscle-type K_{ATP} channels: (1) Are native K_{ATP} channels composed of additional and different regulatory subunits? (2) Do any endogenous ligands regulate K_{ATP} channels? (3) What are the physiological roles of membrane phospholipids (e.g. phosphatidylinositol 4,5-bisphosphates (PIP_2)) in the control of native K_{ATP} channels? (4) What is the localized distribution of the subunits of Kir6.x and SUR.x in a range of smooth muscles? (5) What are the components of native K_{ATP} channels, and how does the stoichiometry of Kir6.x and SUR.x proteins vary between differing smooth muscles? Further studies are required to understand the full complexity of native K_{ATP} channels in smooth muscle.

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